Analysis of geosmin and 2-methylisoborneol in the Regina water supply, July-September, 1984.

A report to the City of Regina and the Saskatchewan Department of the Environment.

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Summary

This work was undertaken with the general purpose of increasing the capability of the Inland Waters Directorate to identify and analyze taste- and odour-causing compounds in water supplies and natural waters. The specific goals were to determine if geosmin and/or 2-methylisoborneol were present in the City of Regina water supply during an odour event, and to determine the temporal variations in their levels. The City was also interested in the spatial variations in the concentration of these compounds as a test for odour production within the distribution system.

Quantitative analyses for geosmin and 2-methylisoborneol were carried out weekly from July 23 to September 24, 1984 on water from 12 locations in the Regina distribution system, and on raw and finished water from the Buffalo Pound Water Treatment Plant. 2-Methylisoborneol was not detected in any of the samples whereas geosmin was detected in most of the samples. It was identified by comparison of gas chromatographic retention time and mass spectrum with geosmin standard. Concentrations varied from less than 0.02 $\mu g\ L^{-1}$ to 1.3 $\mu g\ L^{-1}$. Maximum concentrations occurred in late August. Concentrations within the distribution system were equal to or lower than in the finished water from the treatment plant, with one exception. Thus, this study produced no conclusive evidence for geosmin production within the distribution system. We did observe consistently higher concentrations of geosmin in the finished water than in the raw water for the treatment plant. We have considered two explanations for this: (1) the analytical method gives lower recovery of geosmin from raw water samples, or (2) geosmin is released from (algal) cells which break open during the treatment process. These hypotheses will be tested in 1985.

Introduction

Objectionable taste and odour in water supplies is a serious problem which occurs in many places throughout the world. It may result from industrial contamination or by the (natural) process of eutrophication (Painter et al. 1985). The latter situation is common in western Canada where municipal water supplies are often taken from highly eutrophic lakes, rivers and reservoirs. Actinomycetes bacteria and blue-green algae can produce organic compounds which impart a taste and/or odour to the water (Gerber 1983; Slater and Blok 1983a). The two most common compounds are geosmin (trans,trans-1,10,-dimethyl-9-decalol) and 2-methylisoborneol (MIB, 1,2,7,7-tetramethyl-exo-bicyclo[2.2.1]heptan-2-ol).

The cities of Regina and Moose Jaw, Saskatchewan use Buffalo Pound Lake as a source of drinking water. The water is treated at the Buffalo Pound Water Treatment Plant (BPWTP) and piped to the two cities. There is a serious taste and odour problem with the water most summers (Slater and Blok 1983b), and the situation has received adverse publicity (Maychak 1984; The Globe and Mail 1984). These cities have the highest percentage of point-of-use water filters of any Canadian cities (Tobin 1983). The strategy adopted to reduce or remove the taste and odour is granular activated carbon (GAC) filtration. The treatment plant is scheduled to begin operation in mid-1985. Because of the startup of the GAC plant and the possibility that taste and odour compounds are produced within the distribution system itself, the cities initiated a thorough chemical and microbiological sampling program for their water distribution systems. Sampling began in the spring of 1984 and is still in progress.

At the National Water Research Institute (NWRI) we had begun research into a similar problem for communities along western

Lake Ontario. We identified geosmin in the Burlington water supply during an odour event in August, 1983 (Brownlee et al. 1984). We were unable to establish whether the site of geosmin production was Lake Ontario or the water treatment plant. This initial forray into taste and odour research convinced us of the need for an expanded research effort to identify taste- and odour-causing compounds and establish the mechanism of their production. We are attempting to fill the knowledge gap in this area of water management research. Accordingly, when the opportunity arose to participate jointly with Water Quality Branch (WNR) in the Regina/Moose Jaw study, we saw it as an excellent chance to broaden our research effort while providing information of practical benefit to the cities of Regina and Moose Jaw, the Saskatchewan Department of the Environment, and Environment Canada which is partially subsidizing the construction costs of the GAC plant. The first part of the study will provide baseline information against which to measure the performance of the GAC plant.

Experimental

All organic solvents used in this study were distilled-in-glass and were supplied by Caledon Laboratories, Georgetown, Ontario. MIB was synthesized from d-camphor by the method of Wood and Snoeyink (1977) and was 99.5% pure by gas chromatography (gc). Geosmin standard was obtained from the United States Envirnmental Protection Agency, Drinking Water Division, Cincinnati, Ohio. Anhydrous sodium sulfate (16-20 mesh) was supplied by J. T. Baker Chemical Co., Phillipsburg, N. J.. All glassware was cleaned to remove organic contamination by rinsing with hexane or with acetone followed by hexane.

Samples were collected weekly in clean 4 L amber glass bottles with Teflon-lined caps by staff of the City of Regina and the BPWTP. Twelve locations within the city's distribution system were sampled (R1-R12), as well as the raw and finished water at the BPWTP (BPRW and BPFW). A sample of "odour-free" water (BLANK) was obtained from a GAC pilot unit at the BPWTP.

Quality control consisted of two samples of BLANK water to which known concentrations of geosmin and MIB had been added (STDA and STDB) to determine the extraction efficiency (recovery), the blank, and one duplicate chosen at random from BPFW, BPRW, and R1-R12.

Samples were stored at low temperature (ca. 5°C) until extraction. Just before extraction, they were brought to room temperature by placing the bottles in warm water. The standards were prepared by adding 100 µL of a solution of geosmin and MIB (5 ng μL^{-1}) in ethyl alcohol to 4 L of the "odour-free" water and stirring for 10-15 minutes. This gave a concentration of 0.125 µg L^{-1} of MIB and geosmin.

Samples were extracted by stirring with hexane in 2 L volumetric flasks. Magnetic stirring bars were placed in the flasks which were then filled with sample so that the liquid level was 2-3 cm up the neck. Hexane (5 mL) was added from a dispenser and stirring begun. The stirring rate was set to provide a good vortex. Too rapid stirring may produce emulsions. After two hours, stirring was stopped and the layers were let separate for 0.25-0.5 hour. The hexane layer was carefully withdrawn with a Pasteur pipet and passed through a small column (0.4 by 3 cm) of anhydrous sodium sulfate pre-washed with hexane. The extracts were collected in 25 mL amber glass bottles with Teflon-lined screw caps. The extraction was repeated with a second portion of hexane (5 mL), and the extract passed through the same sodium

sulfate column and combined with the first one. The bottles were capped securely and stored at low temperature (ca. 5°C). The extracts were shipped to NWRI for analysis.

The extracts were prepared for analysis by adding an internal standard and reducing them to a small volume. Octadecane (100 µL of a 20 ng μL^{-1} solution in isooctane) was added to the extract in the 25 mL bottle and mixed well. The extract was then transferred to a 15 mL graduated centrifuge tube by means of a Pasteur pipet, and the extract volume was recorded. The extract was reduced to about 1 mL under a gentle stream of purified argon. At this point a further 100 µL of isooctane was added and the volume further reduced to 0.2 mL. The tube was closed with a Teflon-lined screw cap. The extracts were then analyzed by gc. After analysis, the extracts were transferred to 1.5 mL amber vials for storage.

The extracts were analyzed quantitatively and qualitatively for geosmin and MIB by gc using a flame ionization detector. For quantitative analysis, a Hewlett-Packard model 5710 instrument equipped with a 12.5 m cross-linked dimethyl silicone capillary column (0.22 mm i.d.) was used in split mode (1:10 split ratio). It was programmed from 80-260°C at 4°C $min.^{-1}$. The helium carrier gas flow was 0.70 mL $min.^{-1}$. Under these conditions, the retention times were: MIB, 6.3 min.; geosmin, 12.2 min.; octadecane, 24.4 min.. Injector and detector temperatures were 250 and 300°C, respectively. Peak areas were determined with a Hewlett-Packard model 3392 integrator. Sample size was 3 µL. The detection limit was estimated to be 0.02 µg L^{-1}.

For qualitative anlaysis, a Hewlett-Packard model 5720 instrument equipped with a 30 m DB-17 capillary column (0.3 mm i.d.) was used in splitless mode. This mode gave higher sensitivity but poorer precision, so it was only used to confirm

the presence of geosmin in samples by retention time comparison with standard. The column was programmed from 80-260°C at 4°C $min.^{-1}$ after a 30 s delay. The helium carrier gas flow was 0.9 mL $min.^{-1}$. Retention times were: MIB, 5.8 min.; geosmin, 11.7 min.; octadecane, 23.9 min.. Injector and detector temperatures were 200 and 260°C. Peak areas were determined with a Hewlett-Packard model 3380 integrator. Sample size was 3 µL. Approximately half of the samples were analyzed in this way. The sample was first run by itself and then a mixture of sample and geosmin standard was co-injected. Enhancement of the peak attributed to geosmin relative to the other peaks in the chromatogram was further evidence for geosmin.

Each day, a mixed standard of MIB, geosmin and octadecane was run to establish retention times and to calculate response factors for MIB and geosmin relative to octadecane. With each series, the two "spiked" samples (STDA and STDB) provided the recovery results. Concentrations in the samples were calculated from the peak areas of geosmin relative to octadecane, corrected for response factor and recovery. The BLANK samples were free from MIB and geosmin confirming that the "odour-free" water was suitable for the "spiked" standards.

Each extract was tested for odour at the time of anlaysis by placing ca. 1 µL on a tissue, waiting for the solvent to evaporate, and then smelling the residue.

Geosmin was confirmed by gas chromatography-mass spectrometry (gc-ms). These analyses were performed by the National Laboratory of Water Quality Branch at NWRI on a Finnigan model 4500 instrument operating in the positive ion, electron impact mode.

Results and Discussion

MIB was not detected in any of the samples. The odour of each extract was determined by smelling a small aliquot at analysis time. In many of the samples there was a disinct odour of geosmin. All of these had measurable levels of geosmin. Other samples had complex odours and most of these had geosmin as well. The "odour-free" water from the GAC pilot unit was consistently odour-free.

The identity of geosmin was established by the excellent retention time match in the quantitative chromatograms. Under the qualitative qc conditions retention times were less reproducible, so the geosmin peak was confirmed by peak enhancement by co-injection with geosmin standard. The most conclusive identification of geosmin is the excellent agreement of geosmin spectra from samples with that of the standard (Fig. 1). For the mass spectral work, raw, finished and R9 samples from August 8 were chosen as being "typical" samples. Taken all together, this evidence firmly established that we were indeed measuring geosmin in these samples.

An important aspect of the quantitative analysis was the accuracy and precision of the results, since part of the purpose was to compare concentrations between sampling locations. By doing recovery experiments under conditions closely approximating the actual samples, we were able to partially correct for systematic errors (accuracy). These results are presented in Table 1. We encountered two sources of difficulty with the recovery experiments. After Aug. 27, recovery values were unreliable due to evaporation of the standard. Recoveries up to that time are reliable, but there is a large spread in the values. Rather than use the individual values for each week, recovery values were averaged (N=14, two outliers were left out)

and this average value of 53% ± 7% was used throughout. In retrospect, the concentration of geosmin used in the "spiked" samples, 0.125 μg L^{-1}, was probably too low. A later experiment at 0.25 μg L^{-1} gave an average recovery of 63% ± 6% (n=5), indicating a trend to better recovery at higher geosmin concentrations (and their possible overestimation).

The second aspect of quality control which we attempted to address was random errors (precision). By including one duplicate sample with each series of weekly samples, we had hoped to get a good estimate of the reproducibility of the method. These results are summarized in Table 2 for duplicates which had non-zero values. They are difficult to interpret because precision between duplicates varied over a wide range, and the number of duplicates is too small for a systematic analysis. The main source of error seems to be the sample extraction and preparation stages. The error for gc anlaysis is typically ± 3%. By inspecting the results in Table 2, the error limits seem to be 10-20% with a small probability that it is much larger (50%). With this in mind, sample-to-sample comparisons will be less reliable than set-to-set comparisons.

Geosmin concentrations for all samples are summarized in Table 3, and Figs. 2-6. For all sites, the temporal pattern was similar. There was no geosmin in the July 23 series. Starting with July 30, geosmin began to appear and concentrations increased until late August with values in excess of 1 μg L^{-1} in some samples. Geosmin levels then declined to 0.1 μg L^{-1} or less by late September. This pattern is consistent with anecdotal accounts of the odour event. The threshold odour concentration (TOC) of geosmin is 0.02-0.2 μg L^{-1} (Krasner et al. 1983; Persson 1980). Using the lower value, threshold odour numbers (TON) of about 1-60 would be predicted, and using the upper value the TON's would be 1-6 if geosmin were the sole

source of odour in the samples. The TON values obtained by the City of Regina (L. D. Schnell personal communication) were 4-10 with the higher values occurring at about the same time as geosmin levels were high. Our results predict a much lower minimum TON than obtained by the City. The hexane extraction which we used is selective for nonpolar organic compounds. Other substances not extractable by hexane must produce a background odour in the water.

The other matter of concern is the variation of geosmin levels between the different sites. Sites R1-R12 are served by Buffalo Pound water or by groundwater or by mixtures of these. Site R10 is groundwater only and never had detectable geosmin. All the other sites had measurable levels of geosmin. The highest values were for R1-R4. These sites are in that part of the distribution system closest to the source (BPFW). Only R2 had a mean concentration higher than BPFW. This would indicate possible production of geosmin in that part of the distribution system. To further examine this possibility, the results from R2 were compared with those from BPFW by quadratic and linear regression methods (A. H. El-Shaarawi personal communication). The quadratic regressions are shown in Fig. 7 for $Y = b_0 + b_1X + b_2X^2$, where Y is geosmin concentration and X is the week of sampling. From these results and from linear regression of R2 values against BPFW (Fig. 8a), we estimate that there is at least a 60% probability that concentrations at R2 are significantly higher than in the finished water. It is possible, but not certain, that geosmin is being produced in the distribution system at this location by microbial action or cell lysis following chlorination. There is no evidence for geosmin production at any of the other locations sampled.

Comparison of geosmin levels in the raw and finished water (Fig. 8b) shows an unexpected difference. Raw water concentrations

were about 30% of finished water concentrations. It is possible, but not likely, that our extraction method was less efficient for dissolved geosmin in raw water. Because of this, alternative extraction methods will be tried in 1985. A better explanation is that geosmin is released from algal cells during the water treatment process, resulting in higher levels of dissolved geosmin in the finished water. If this is so, then the algal cells should contain appreciable levels of geosmin. This will also be investigated in 1985.

Acknowledgements

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Figure Legend

Figure 1. Mass spectra of geosmin in raw water, finished water, and R9 samples from August 8, 1984, and geosmin standard.

Figure 2. Geosmin concentrations in raw and finished water.

Figure 3. Geosmin concentrations in water from R1, R2 and R3.

Figure 4. Geosmin concentrations in water from R4, R5 and R6.

Figure 5. Geosmin concentrations in water from R7, R8 and R9.

Figure 6. Geosmin concentrations in water from R11 and R12.

Figure 7. Quadratic regressions for results from R2 and finished water.

Figure 8. Linear regressions for results from (a) R2 vs. finished water, and (b) finished water vs. raw water.

Table 1. Per cent recoveries for geosmin.

Date	Sample	Recovery Per Cent
7/17	STD1-A	52.6
	STD1-B	66.8
7/18	STDA	55.1
	STDB	48.2
7/23	STDA	54.6
	STDB	51.8
7/30	STDA	57.7
	STDB	53.6
8/8	STDA	36.3
	STDB	60.9
8/13	STDA	52.4
8/20	STDA	53.7
	STDB	50.6
8/27	STDB	44.7
	Mean	52.8
	Std. Dev.	6.9

STD1-A and STD1-B were standards with 0.25 µg L^{-1} of geosmin.

STDA and STDB were standards with 0.125 µg L^{-1} of geosmin.

Table 2. Geosmin concentrations (μg L^{-1}) for duplicate samples.

Date	Sample	Geosmin	Mean	Difference	Ratio Diff/Mean
7/30	R8A	0.021	0.020	0.002	0.10
	R8B	0.019			
8/8	R1A	0.384	0.371	0.027	0.07
	R1B	0.357			
8/13	R5A	0.316	0.287	0.058	0.20
	R5B	0.258			
8/20	BPRW-1	0.051	0.062	0.022	0.36
	BPRW-2	0.073			
8/27	R7A	0.837	0.785	0.105	0.13
	R7B	0.732			
9/17	R4A	0.157	0.216	0.117	0.54
	R4B	0.274			

Table 3. Geosmin concentrations ($\mu q\ L^{-1}$) for July 23 to Sept. 24, 1984.

Sample	Date 7/23	7/30	8/8	8/13	8/20	8/27	9/4	9/11	9/17	9/24	Mean	Std. Dev.
R1	0.00	0.08	0.37	0.57	1.03	1.09	0.54	0.45	0.16	0.06	0.43	0.37
R2	0.00	0.04	0.55	1.14	1.02	1.29	0.88	0.90	0.10	0.12	0.60	0.48
R3	0.00	0.05	0.54	0.65	0.84	0.79	0.51	0.56	0.18	0.10	0.42	0.30
R4	0.00	0.04	0.37	1.14	0.45	0.57	0.31	0.40	0.22	0.07	0.36	0.32
R5	0.00	0.03	0.14	0.29	0.35	0.47	0.64	0.36	0.18	0.07	0.25	0.20
R6	0.00	0.00	0.17	0.39	0.29	0.32	0.11	0.67	0.06	0.03	0.20	0.20
R7	0.00	0.02	0.17	0.08	0.00	0.22	0.29	0.64	0.05	0.00	0.15	0.19
R8	0.00	0.02	0.11	0.50	0.99	0.73	0.36	0.05	0.06	0.05	0.29	0.33
R9	0.00	0.06	0.49	0.60	0.73	0.57	0.55	0.64	0.19	0.08	0.39	0.26
R10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R11	0.00	0.05	0.52	0.65	0.57	0.68	0.30	0.58	0.22	0.10	0.37	0.25
R12	0.00	0.05	0.40	0.58	0.64	0.46	0.34	0.33	0.08	0.02	0.29	0.23
BPRW	0.00	0.09	0.28	0.25	0.06	0.20	0.08	0.18		0.00	0.13	0.10
BPFW	0.00	0.15	0.83	0.56	0.75	1.06	0.66	0.52	0.09	0.10	0.47	0.35
BLANK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		

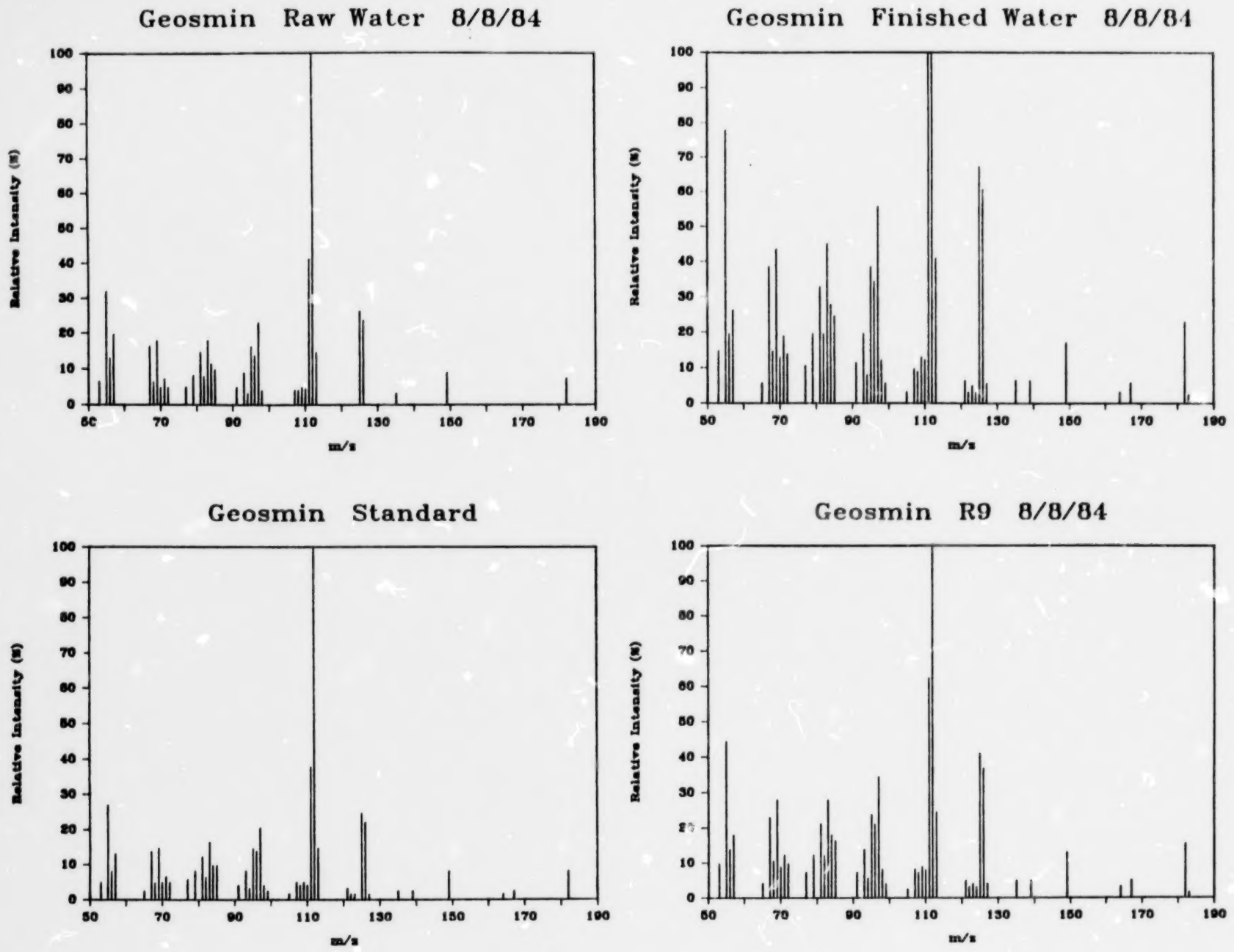

Geosmin Raw Water 8/8/84
Geosmin Finished Water 8/8/84
Geosmin Standard
Geosmin R9 8/8/84
Relative Intensity (%)
m/z
50
70
90
110
130
150
170
190
0
10
20
30
40
50
60
70
80
90
100

Figure 1.

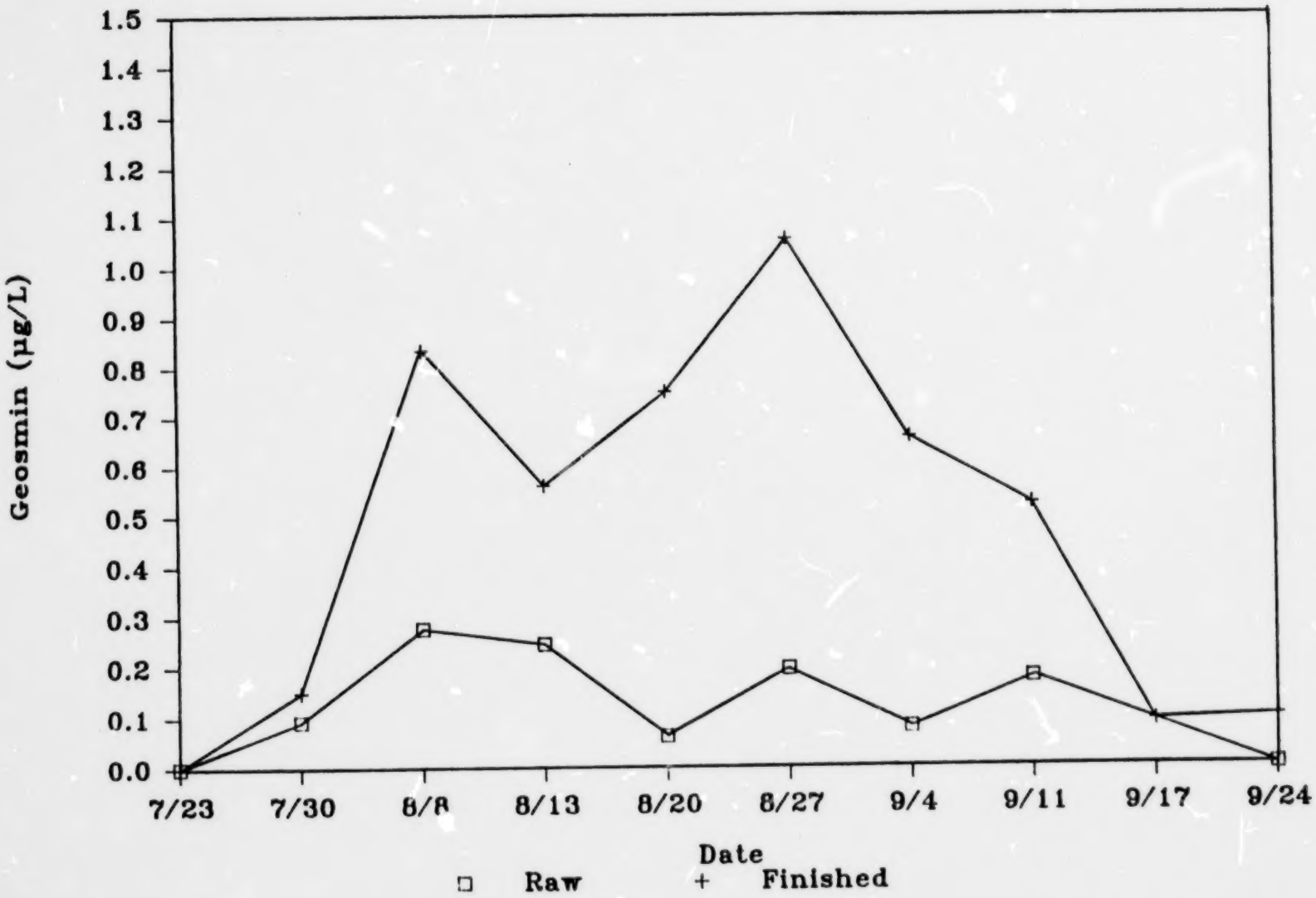

Raw and Finished
Geosmin (µg/L)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
7/23
7/30
8/6
8/13
8/20
8/27
9/4
9/11
9/17
9/24
Date
Raw
Finished

Figure 2.

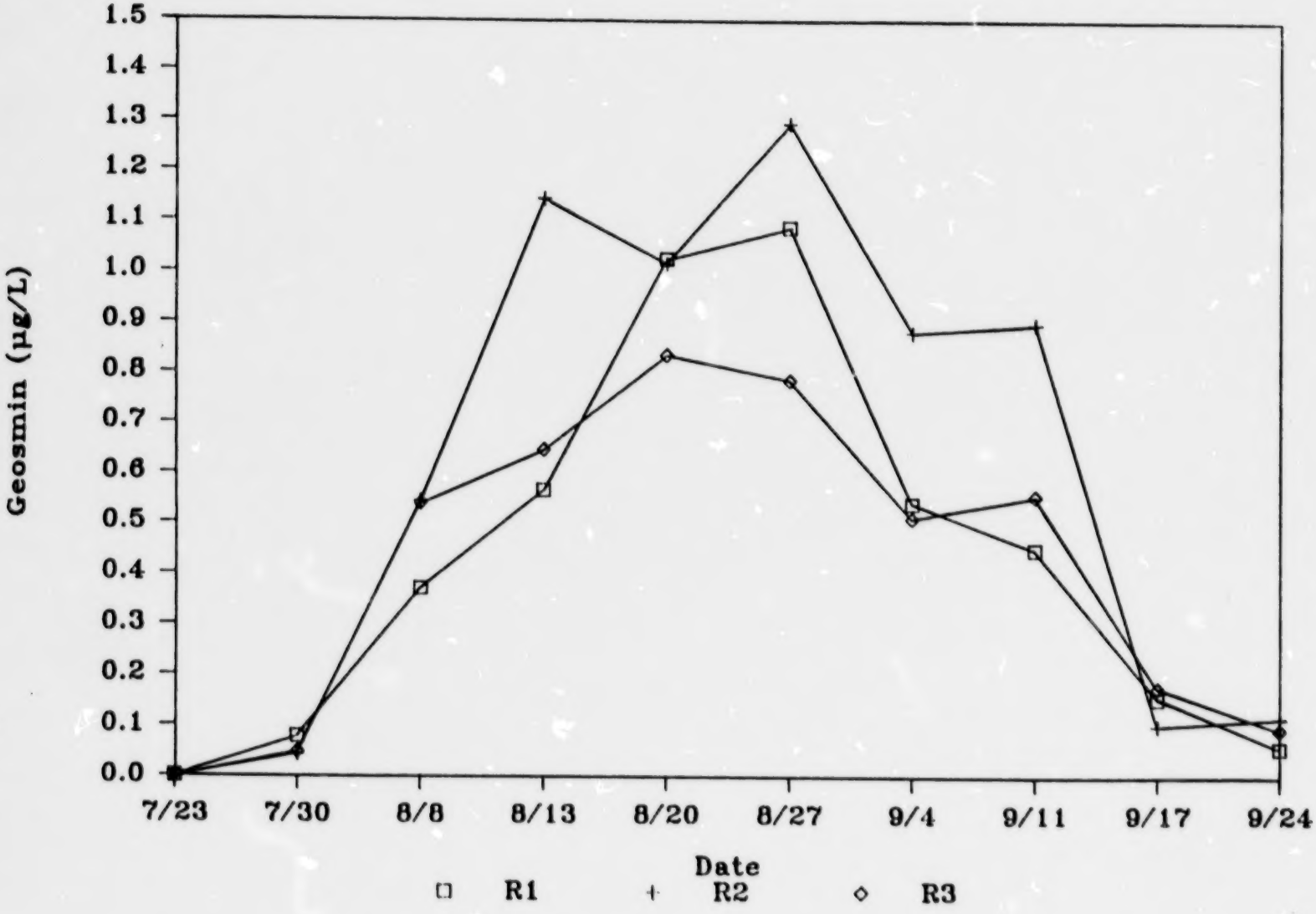

R1 to R3
Geosmin (µg/L)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
7/23
7/30
8/6
8/13
8/20
8/27
9/4
9/11
9/17
9/24
Date
R1
R2
R3

Figure 3.

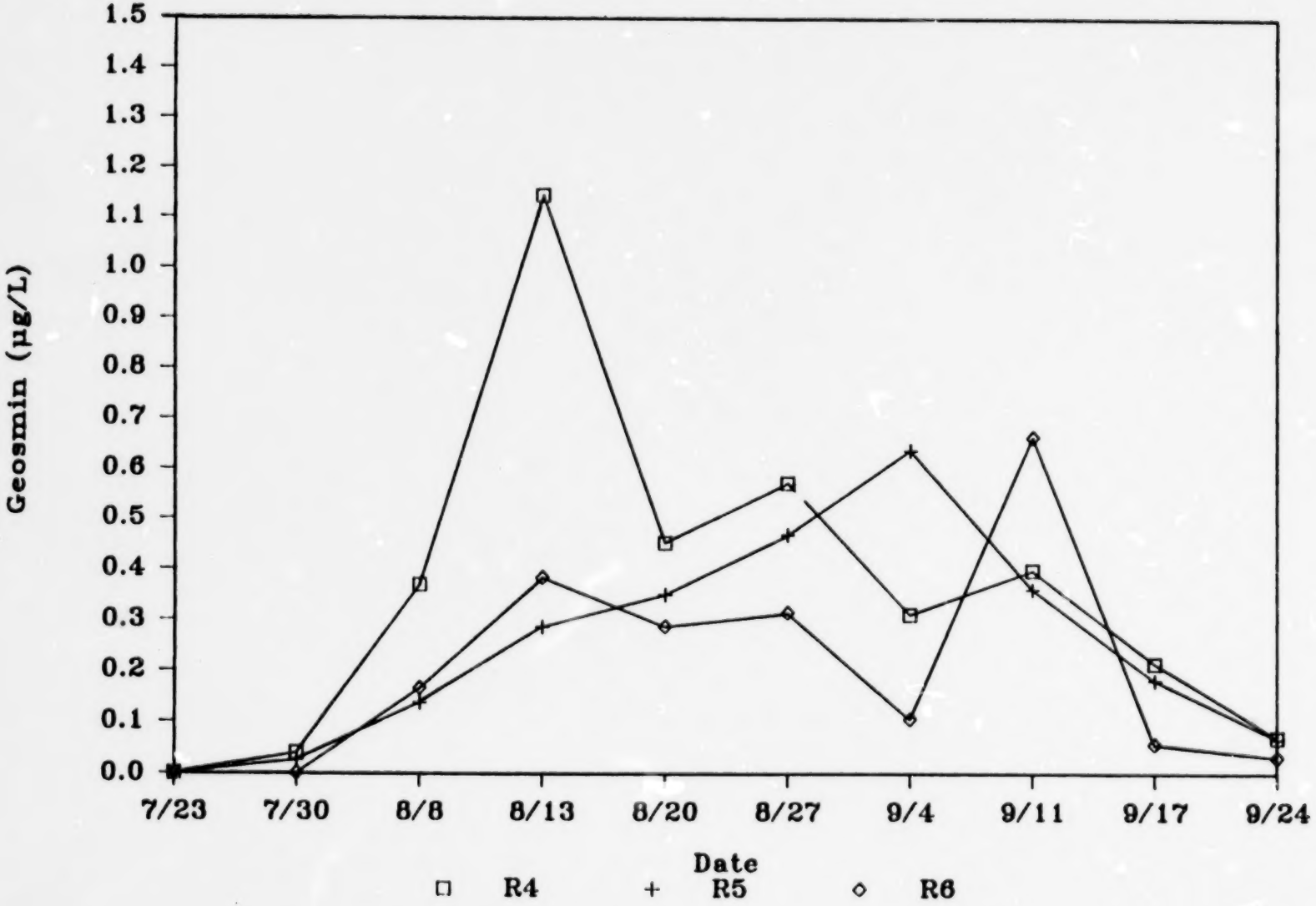

R4 to R6
Geosmin (µg/L)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
7/23
7/30
8/6
8/13
8/20
8/27
9/4
9/11
9/17
9/24
Date
R4
R5
R6

Figure 4.

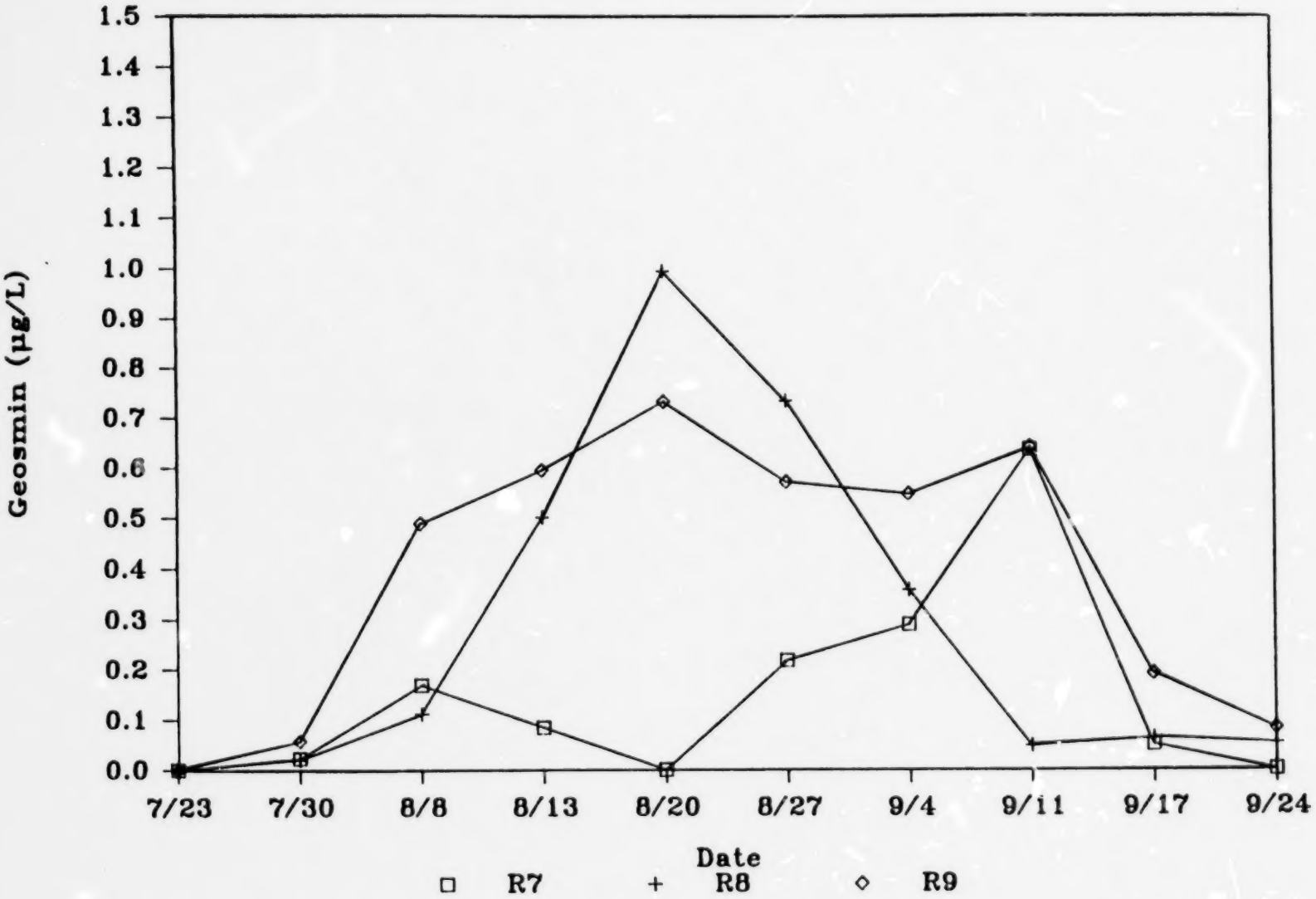

R7 to R9
Geosmin (µg/L)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
7/23
7/30
8/6
8/13
8/20
8/27
9/4
9/11
9/17
9/24
Date
R7
R8
R9

Figure 5.

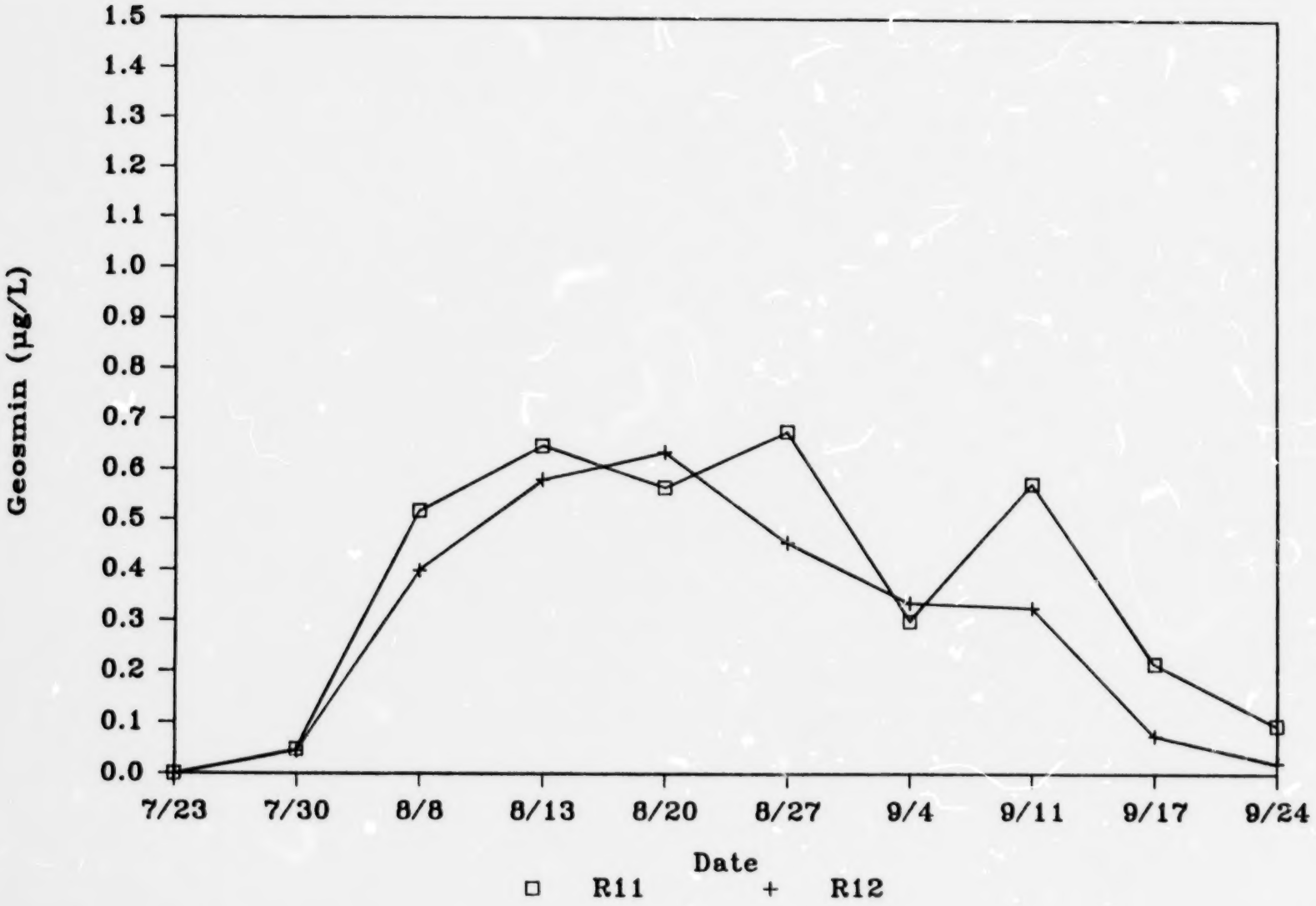
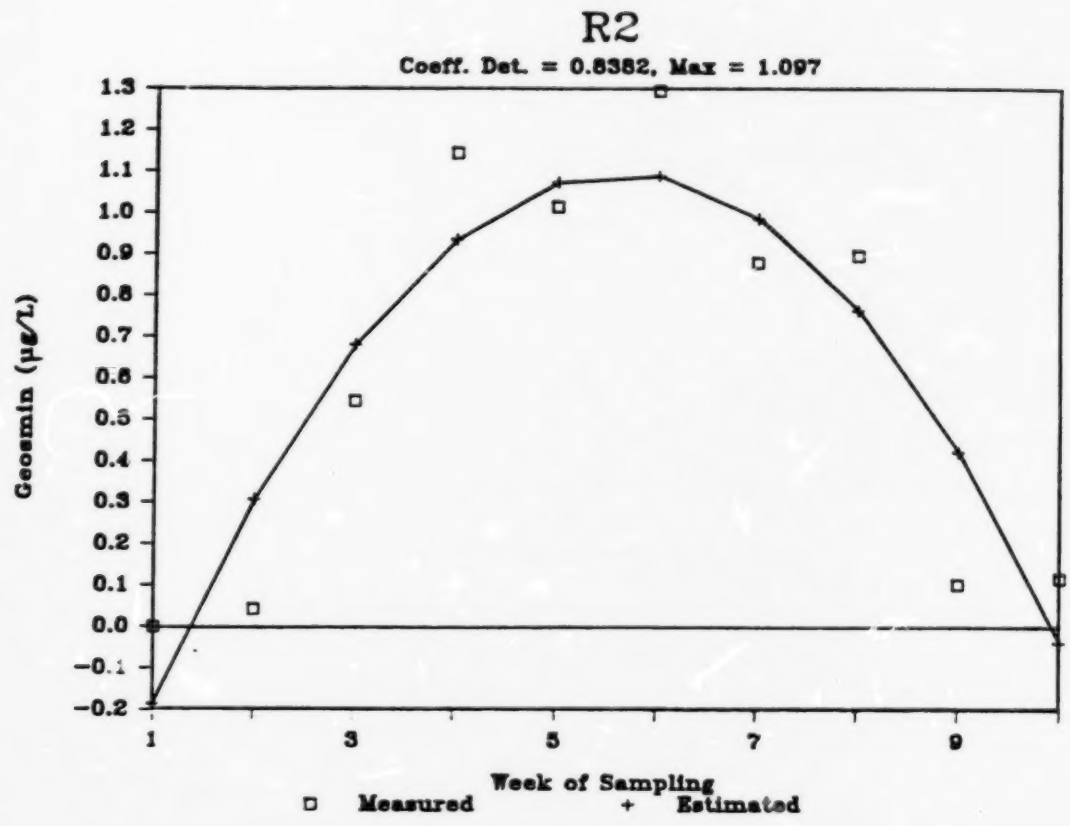

R11 and R12
Geosmin (µg/L)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
7/23
7/30
8/6
8/13
8/20
8/27
9/4
9/11
9/17
9/24
Date
R11
R12

Figure 6.

Figure 7.


R2
Coeff. Det. = 0.8382, Max = 1.097
Geosmin (µg/L)
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
1
3
5
7
9
Week of Sampling
Measured
Estimated

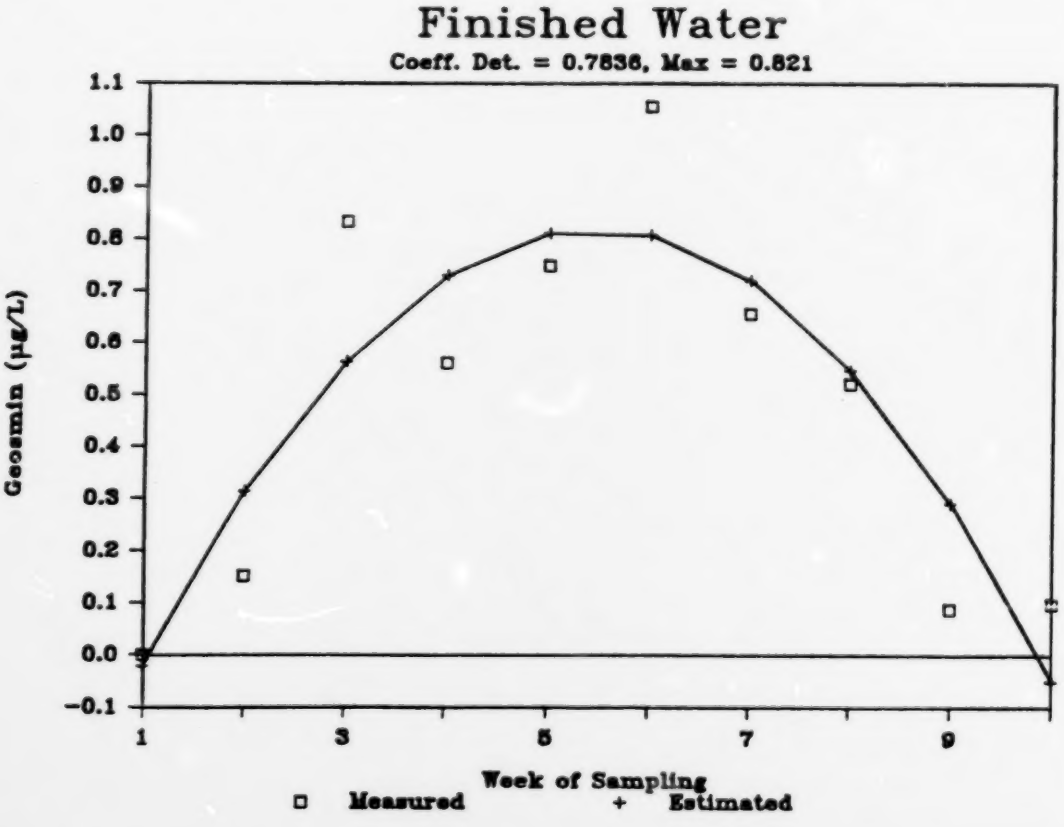
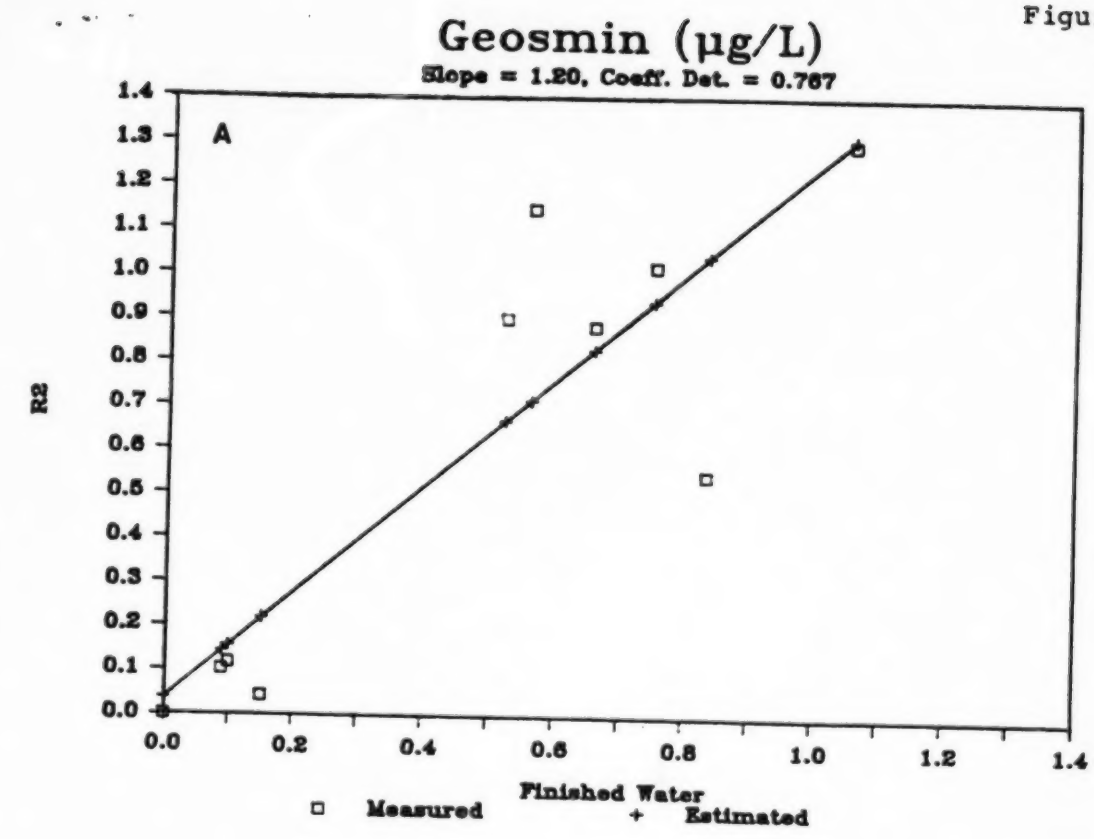
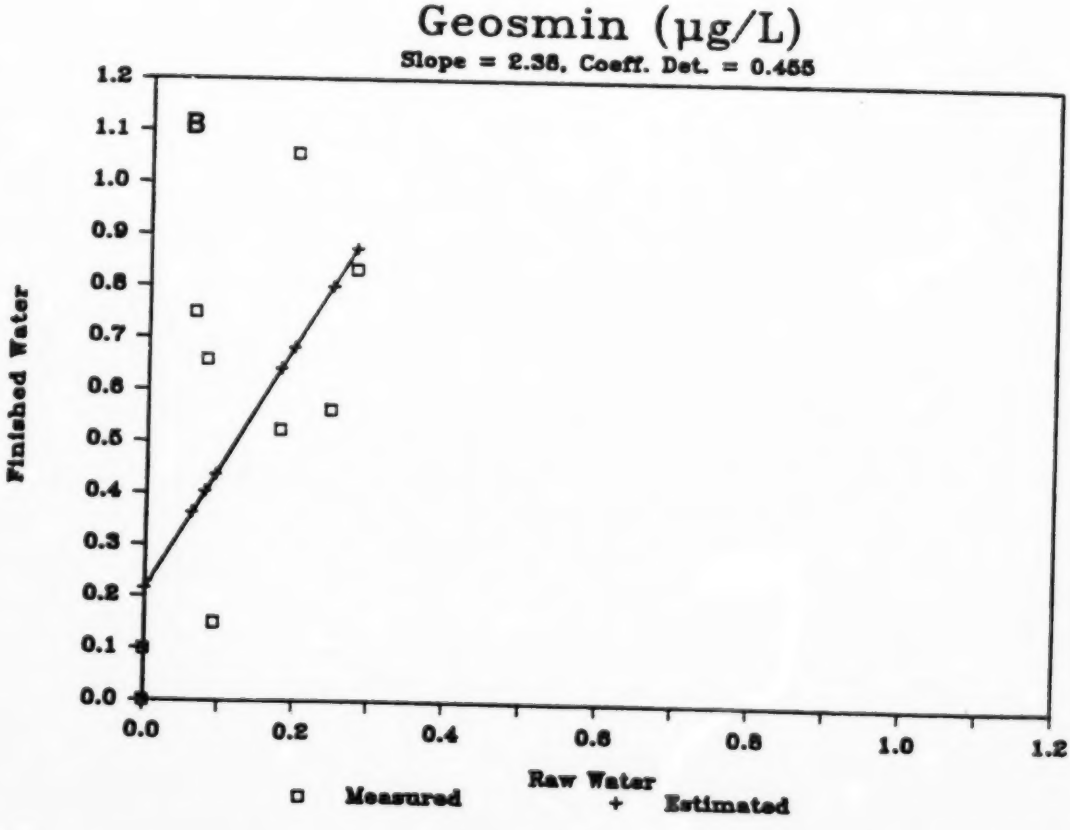

Finished Water
Coeff. Det. = 0.7836, Max = 0.821
Geosmin (µg/L)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
1
3
5
7
9
Week of Sampling
Measured
Estimated

Figure 8.


Geosmin (µg/L)
Slope = 1.20, Coeff. Det. = 0.767
A
R2
Finished Water
Measured
Estimated


Geosmin (µg/L)
Slope = 2.36, Coeff. Det. = 0.455
B
Finished Water
Raw Water
Measured
Estimated